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Level of α-Catenin Expression in Colorectal Cancer Correlates With Invasiveness, Metastatic Potential, and Survival

IOANNIS RAFTOPOULOS, MD,[1]* PANAGIOTIS DAVARIS, MD, PhD,[3]
GABRIEL KARATZAS, MD, PhD,[1] PANAGIOTIS KARAYANNACOS, MD, PhD,[2] AND
GREGORY KOURAKLIS, MD, PhD[1]**

[1]*Second Department of Propedeutic Surgery, Laikon Hospital, Athens University Medical School, Athens, Greece*
[2]*Laboratory of Experimental Surgery and Surgical Research, Athens University Medical School, Athens, Greece*
[3]*Department of Pathology, Athens University Medical School, Athens, Greece*

Background and Objectives: Decreased expression of the E-cadherin/α-catenin cell–cell adhesion complex is considered to elicit detachment of tumor cells from primary lesions and development of metastases. The immunohistochemical profile of α-catenin in colorectal cancer, as well as its correlation with differentiation, lymph node/liver metastasis and patient survival is presented in this study.
Methods: α-Catenin expression was investigated with immunohistochemistry technique, in 85 paraffin-embedded and 21 fresh frozen specimens, including 82 colon adenocarcinomas, 10 adenomas, 10 lymph nodes, and 3 liver metastases. Preserved α-catenin expression was considered for those tumors that demonstrated more than 90% α-catenin(+) cancer cells and reduced α-catenin expression for those tumors with less than 90% α-catenin(+) cancer cells. The χ^2-test was used to calculate the statistical correlation of α-catenin expression with grade of differentiation and metastatic potential and the log-rank test for the correlation with survival rate.
Results: Normal mucosa, as well as 8/10 of the colon adenomas, showed strong membranous α-catenin expression. Reduced α-catenin expression was found in 32/82 (39%) colorectal cancers examined, which was associated with de-differentiation ($P < 0.01$), lymph node metastasis ($P < 0.025$), and poor clinical outcome ($P < 0.012$). α-Catenin expression was preserved in 3 liver metastases and their corresponding primary tumors. By contrast, 6/10 of lymphogenous metastases showed decreased α-catenin expression.
Conclusions: Our findings demonstrate a significant down-regulation of α-catenin expression in colorectal cancer which is associated with poor differentiation, higher metastatic potential and unfavorable prognosis. These preliminary results suggest that α-catenin may be a useful marker of invasiveness, metastatic potential, and survival in colorectal cancer patients. *J. Surg. Oncol. 1998;68:92–99.*

KEY WORDS: α-catenin; colorectal cancer; differentiation; lymph node metastases; liver metastases; prognosis

INTRODUCTION

The progression of colorectal cancer, either locally by direct invasion through the bowel wall into adjacent structures, or by metastasis, through lymphatic and ve-

*Correspondence to: Ioannis Raftopoulos, MD, 856 West Nelson St., Apt. 1002, Chicago, IL 60657; or **Gregory Kouraklis, MD, FACS, Second Department of Propedeutic Surgery, Laikon Hospital, Athens University Medical School, 122 Vasilissis Sofias Avenue, 11526 Athens, Greece.

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nous channels, to regional lymph nodes and distant organs, involves ability of malignant cells to modulate their intercellular connections and to detach from the primary tumor [1]. The E-cadherin cell adhesion complex is responsible for intercellular adhesion and plays a fundamental role in maintaining the epithelial cell phenotype [2]. The E-cadherin is expressed in all normal epithelial cells and is often down-regulated in various cancers [3]. Previous studies have shown that decreased E-cadherin expression occurs frequently in colorectal cancer and is statistically correlated with poor differentiation [4], advanced Dukes stage [5], and poor survival [6].

It was recently reported that E-cadherin is linked to the cytoskeleton with three cytoplasmic proteins called catenins (α-, β- and γ-catenin) [7]. E-cadherin interacts directly with β- or γ-catenin, whereas α-catenin binds indirectly to E-cadherin via the other catenins and mediates the connection of E-cadherin cell adhesion complex with the actin cytoskeleton [2]. β-Catenin, apart from linking with E-cadherin, interacts with the epidermal growth factor receptor and becomes tyrosine phosphorylated [8]. Recently, it was reported that β-catenin associates with the adenomatous polyposis coli (APC) gene product [9].

Previous studies suggest that α-catenin expression is reduced in esophageal [10], gastric [11], and breast cancer [12] and, in comparison with E-cadherin expression, it is more closely correlated with invasive phenotype and lymph node metastasis. On the other hand, decreased expression of α-catenin in head and neck squamous cell carcinomas did not correlate with de-differentiation, metastasis, or poor survival [13].

Controversy exists over the role of α-catenin in colorectal cancer. One previously published study showed correlation of decreased α-catenin expression with poor differentiation and presence of metastases [14]. Other studies failed to find a significant association of reduced α-catenin and p120-catenin expression with any of the clinicopathologic parameters [15–18]. In addition, the immunohistochemical profile of α-catenin has not been determined previously in colorectal cancer in relation to patient survival.

Furthermore, in an effort to further elucidate the molecular interactions that occur during the malignant transformation and the metastasis of colorectal cancer, we investigated the expression of α-catenin in precancerous adenomas, as well as in paired samples of lymph node and liver metastases and their corresponding primary tumors.

MATERIALS AND METHODS

Tissues

A total of 85 paraffin-embedded sections from 72 patients were included in the study. Forty-nine colorectal carcinomas, 10 adenomas, 10 lymph node metastases, and 3 liver metastases, as well as the paraffin blocks from the 13 corresponding primary tumors, were provided by the Department of Pathology, Athens University Medical School. A further 21 fresh frozen specimens, including 20 colorectal adenocarcinomas and 1 peritoneal metastasis, were also obtained by the Second Department of Propedeutic Surgery, Laikon Hospital, Athens University Medical School.

In addition, paraffin-embedded and fresh frozen samples of normal colonic mucosa were also made available, in order to compare α-catenin expression in formalin-fixed paraffin-embedded and fresh frozen tissues. None of the patients received anticancer therapy preoperatively.

Materials

The polyclonal rabbit anti-α-catenin antibody (Sigma Bio Sciences, St. Louis, MO) was used at a dilution of 1:1,000 in pH 7.6 Tris-buffered saline (TBS). The antibody was raised in rabbit against a synthetic peptide that corresponds to amino acids 890–901 of human/mouse α-catenin. Immunostaining was performed using the Streptavidin ABC/ HRP Duet, Mouse/Rabbit Kit (Dako, High Wycombe, UK) at a dilution of 1:100 in TBS. The optimal dilution for anti-α-catenin antibody for paraffin-embedded and frozen sections was established in preliminary experiments.

Immunohistochemistry

In this study, 5-μm-thick paraffin sections were cut, attached to glass slides, and then dewaxed, hydrated, and incubated with 1% hydrogen peroxide in distilled water for 10 min to inhibit endogenous peroxidase activity. The microwave antigen retrieval method was used then, in order to enhance α-catenin expression in formalin-fixed (10% neutral buffered formalin) paraffin-embedded tissues [19]. Briefly the slides were submerged in a plastic Coplin jar, filled with citrate buffer (2.1 g citric acid monohydrate in 1 L of distilled water, pH 6.0), and covered with perforated paper to minimize evaporation. The slides were then heated in a 700-W microwave oven on full power for 5 × 2-min cycles pausing to ensure there was no fluid loss due to evaporation. Slides were then allowed to cool at room temperature for 10 min and incubated with the primary antibody at 4°C overnight. The specimens were sequentially incubated with the biotinylated goat antibody to mouse/rabbit IgGs and the Strept ABComplex reagents for 30 min at room temperature. Visualization was achieved with 100 μl of diaminobenzidine (DAB) (Dako) at a concentration of 1 mg/10 ml, supplemented with 0.04% hydrogen peroxide for 15 min, and counterstained with Harris's hematoxylin

(Merck, Darmstadt, Germany). The slides were rinsed in TBS (×3) after each stage.

The fresh tissues were immediately (up to 30 min after surgical removal) snap frozen and stored at −70°C until sectioning. The sections were cut at 5-μm, attached to slides and fixed in acetone at −20°C for 10 min. The specimens were treated with 1% hydrogen peroxide in methanol for 10 min, to inhibit endogenous peroxidase activity, and processed as above, omitting the microwave step. The antigen retrieval method was validated in preliminary experiments by demonstrating identical α-catenin staining in consecutive frozen and paraffin sections of the same normal colonic mucosa.

In order to ensure accurate and reproducible staining, normal colonic mucosa was used as positive control. Additionally, normal colonic cells present in the tumor sections were also used as internal positive control. Negative controls were duplicate sections similarly stained, in which the primary antibody was omitted and replaced by either TBS or nonimmune rabbit serum.

Evaluation of α-Catenin Expression

α-Catenin expression was evaluated by light microscopy, by two independent observers with no synchronous knowledge of the tumors' clinicopathologic parameters. The expression of α-catenin was considered positive, when staining of cancer cells was as strong as that of normal colonic cells. When the proportion of α-catenin-positive cancer cells in each section was more or less than 90%, the tumors were evaluated as α-catenin preserved or α-cat(+) and reduced α-cat(−), respectively.

Histologic Evaluation

A consecutive section from each specimen was stained with hematoxylin and eosin (H&E) for histological evaluation.

Statistical Analysis

Statistical correlation of α-catenin expression with grade of differentiation, Dukes stage, and tumor size was analyzed with the χ^2-test. Kaplan-Meier actual statistics based on the life-table method were used to evaluate the relationship between α-catenin expression and disease outcome in 40 of our patients who had a mean follow-up of 32.4 months (follow-up range: 21–49 months, SE: 1.63). Patients who died within 1 month postoperatively or from other causes were excluded. In addition, specimens from cancer patients who refused to participate or could not be contacted were also excluded. The Mantel-Haenszel or log-rank test was used for comparison of survival rates between the group with α-catenin-positive tumors and the group with α-catenin-negative tumors. *P*-values of <0.05 were accepted as statistically significant and were based on two-tailed tests.

RESULTS

Expression of α-Catenin in Normal Colorectal Mucosa and Adenomas

Normal colonic epithelium strongly expressed α-cat at the cell membrane, with similar intensity in both paraffin-embedded and fresh frozen samples (Fig. 1a). In addition, 8 of 10 adenomas examined showed strong, membranic α-cat expression of equal intensity with the normal tissues (Fig. 1b,c). However, two tubular adenomas showed reduced α-cat expression with cytoplasmic distribution (Table I). No correlation was found between loss of α-cat expression in adenomas and grade of dysplasia.

Expression of α-Catenin in Colorectal Cancer

Decreased α-catenin expression was found in 32/82 (39%) colorectal adenocarcinomas, examined. Complete loss of α-cat staining was not found in any of the samples. The majority of α-cat (+) cancer cells formed gland-like aggregates and exhibited tight intercellular adhesion (Fig. 1d). In the 32 tumors with reduced α-catenin expression, 25 showed focal loss of α-cat expression (Fig. 1e), whereas the remaining 7 tumors exhibited diffusely weak staining.

Correlation Between α-Catenin Expression and Clinicopathologic Parameters

The frequency of preserved α-cat expression was higher in well-differentiated carcinomas (16/19, 84%) than in moderately (27/41, 66%), or poorly differentiated carcinomas (4/13, 31%). In addition, preserved α-cat(+) expression was found more frequently in tumors without lymph node metastasis (76.6%, 36/47) than in tumors with lymph node metastasis (40%, 10/25).Thus, reduced α-catenin expression was statistically correlated with poor differentiation ($P < 0.01$) and lymph node metastasis ($P < 0.005$). On the other hand, no correlation was found between reduced α-catenin expression and presence of liver metastasis, or tumor size (Table II).

Correlation Between α-Catenin Expression and Clinical Outcome

We found that down-regulation of α-catenin expression is statistically associated with poor prognosis and poor clinical outcome. The patients with α-cat(+) tumors demonstrated a statistically significant better prognosis ($P < 0.012$) (Fig. 2).

α-Catenin Expression in Lymph Node, Liver and Peritoneal Metastases and the Corresponding Primary Colorectal Tumors

Our study showed that α-catenin expression was reduced in 6/10 (60%) lymph node metastases. By contrast,

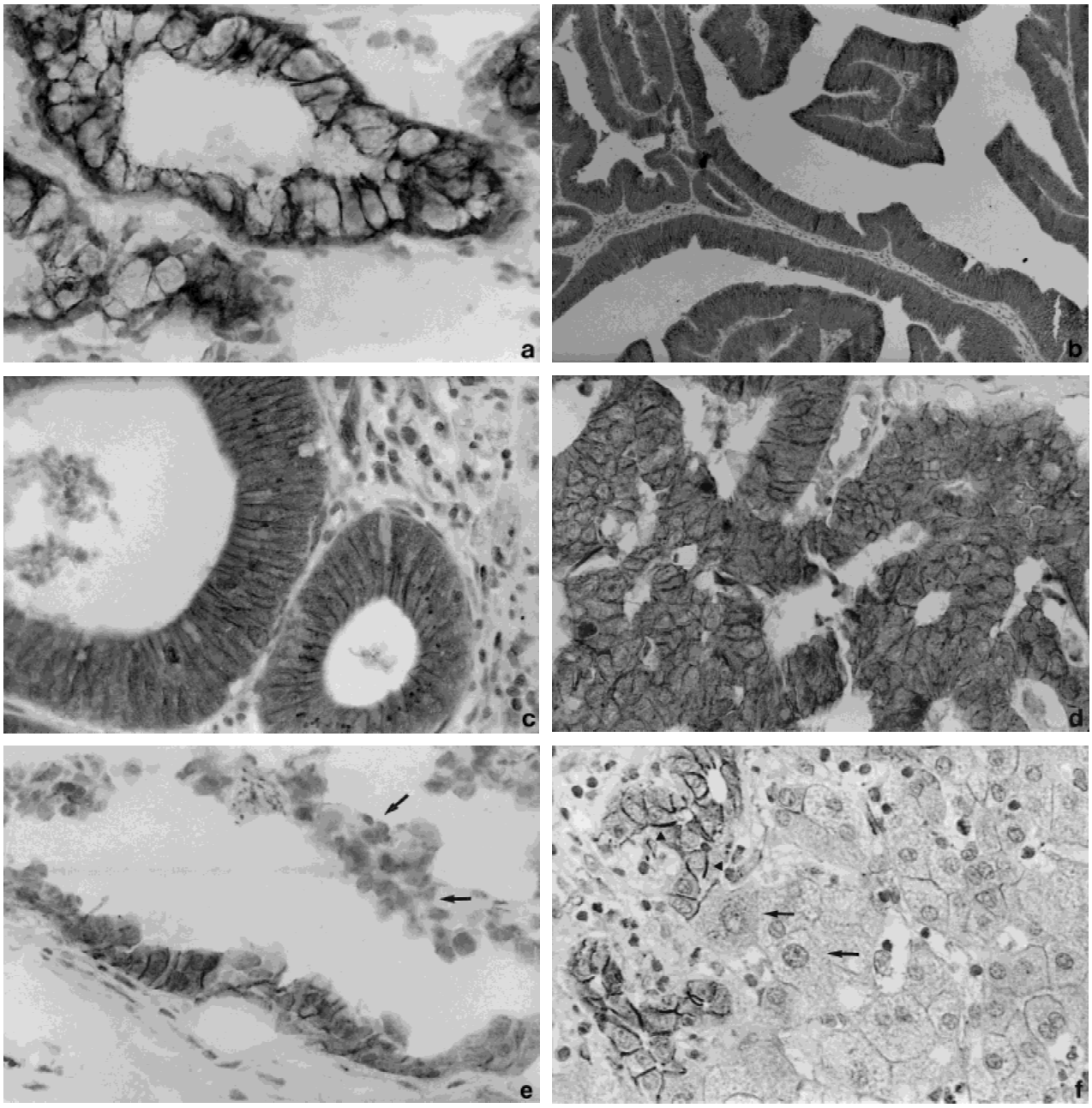

Fig. 1. Detection of α-catenin expression with immunohistochemistry technique. **a:** Cells of noncancerous crypts of colonic mucosa. Noncancerous epithelial cells strongly and homogeneously express α-catenin at cell–cell boundaries. **b, c:** Preserved α-catenin expression in paraffin-embedded colorectal adenomas. **d:** Preserved α-catenin expression in a moderately differentiated paraffin-embedded colorectal adenocarcinoma. **e:** Moderately differentiated colorectal carcinoma illustrating heterogeneity of α-catenin expression. Loss of expression occurs (arrow) in less well differentiated areas. **f:** Paraffin-embedded liver metastasis of a colorectal cancer, which preserves α-catenin expression. *Arrow, arrowhead,* indicate hepatocytes and α-catenin(+) colon cancer cells, respectively. **a-f:** ×500.

all liver metastases (3/3) exhibited strong α-catenin staining (Fig. 1f). No significant difference was found in the staining pattern or intensity between lymph node and liver metastases and primary tumors (Table III). Furthermore, one peritoneal metastasis of a moderately differentiated colorectal carcinoma showed reduced α-cat expression.

DISCUSSION

All normal colorectal epithelial cells of both fresh frozen and paraffin-embedded tissue sections showed equally strong, membranous expression of α-catenin protein at the cell–cell borders, which reflects the normal localization of an intercellular adhesion molecule. Al-

TABLE I. α-Catenin Expression in Colorectal Adenomas

Histologic type	α-Catenin expression (No. of cases)		
	Preserved	Reduced	Total
Tubular adenomas	2	2	4
Mixed adenomas	6	0	6

TABLE II. α-Catenin Expression in Colorectal Cancer*

Clinicopathologic parameters	α-Catenin expression					
	Paraffin		Frozen		Total	
	Pr[a]	Rd[b]	Pr	Rd	Pr/Rd	χ^2/P
Well/moderately differentiated	33	13	10	4	43/17	
Poorly differentiated	4	9	—	—	4/9	<0.01
Dukes stage A + B	28	9	8	2	36/11	
Dukes stage C	7	13	3	2	10/15	<0.025
Dukes stage D	3	1	—	—	3/1	NS

*Statistically significant correlations are referred only to the total number of both paraffin-embedded and fresh frozen tissue specimens.
[a]Pr, preserved α-cat expression.
[b]Rd, reduced α-cat expression.

though the number of specimens examined was small, we demonstrate that α-catenin expression seems to be preserved in colorectal adenomas. Only 2 of 10 adenomas were found to have reduced α-catenin expression with no correlation with the grade of dysplasia.

Our findings are in line with recent studies reporting that α-catenin expression is preserved in the colorectal adenomatous polyps [20]. It is noteworthy, that β-catenin, which is more directly associated with E-cadherin and the APC gene product, was frequently down-regulated [20]. It has also been reported that E-cadherin expression is often decreased in colorectal adenomas and is associated with larger polyp size, higher grade of dysplasia, and villous histology [4,21].

Furthermore, in accordance with a previous study [14], we found a significant reduction of α-catenin expression in 32 of 82 (39%) colorectal carcinomas that we examined, which was statistically associated with poor differentiation and lymph node metastasis. Other studies, however, in colorectal adenocarcinomas, also demonstrate a frequent down-regulation of α-catenin and p120-catenin expression, but without any correlation with poor differentiation, or metastatic potential [15–17]. It should be noted that Shiozaki et al. [16] examined only 10 patients with colorectal cancer. On the other hand, Hugh et al. [17] reported that 25 of the 38 specimens with abnormally scored α-catenin staining showed strong membrane and cytoplasmic expression together. Considering that in this study no association was found between α-catenin expression and differentiation or metastasis, it is unclear whether α-catenin in these cancer cells with combined membrane and cytoplasmic expression was dysfunctional. In contrast with the above, there is one study in the recent literature that finds no significant down-regulation of α-catenin expression in colorectal cancer [18].

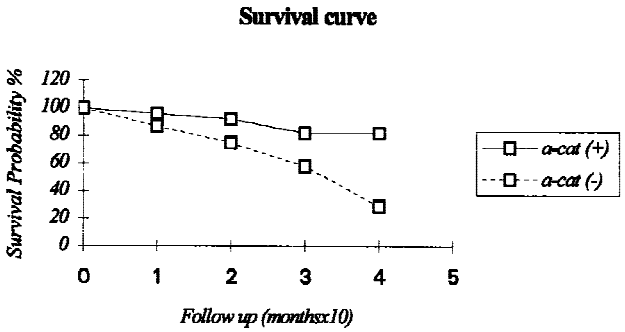

Fig. 2. Survival curve of the patients with preserved [α-cat(+)] and reduced [α-cat(−)] expression, respectively. Statistical significance by log-rank, or Mantel-Haenszel test ($P = 0.012$).

TABLE III. Comparison of α-Catenin Expression in Primary Tumors and the Corresponding Lymph Node and Liver Metastases*

Tumor expression	Lymph node expression		Liver expression	
	Pr α-cat	Rd α-cat	Pr α-cat	Rd α-cat
Pr α-cat	3	1	3	0
Rd α-cat	1	5	0	0
Total	4	6	3	0

*Pr, preserved α-cat expression; Rd, reduced α-cat expression.

In accordance with our results, it has been reported that α-catenin expression is often decreased in gastric [22] and esophageal cancer [10] and is associated with poor differentiation and lymph node metastasis (Fig. 3). These studies have also demonstrated that reduction of α-catenin expression seems to be a better predictor of de-differentiation and lymph node metastasis than E-cadherin. The higher frequency of α-catenin down-regulation reported in gastric and esophageal cancer, in comparison with our study, can be explained by the fact that these tumors are more invasive, have been metastasized more frequently at the time of diagnosis and are associated with worse prognosis than colorectal cancer.

Therefore, α-catenin down-regulation seems to be a more sensitive marker of malignant transformation and metastatic potential, while E-cadherin dysfunction can be observed in premalignant states as well, but is not associated as closely as α-catenin with invasiveness and metastatic spread. It has been shown that the mutual adhesiveness of cancer cells, even when they are bound to one another, is weak and the junctional structures that they form is frequently insufficient [23]. It is possible that dysfunction of E-cadherin and β-catenin, which are directly linked to each other, might change the adhesive-

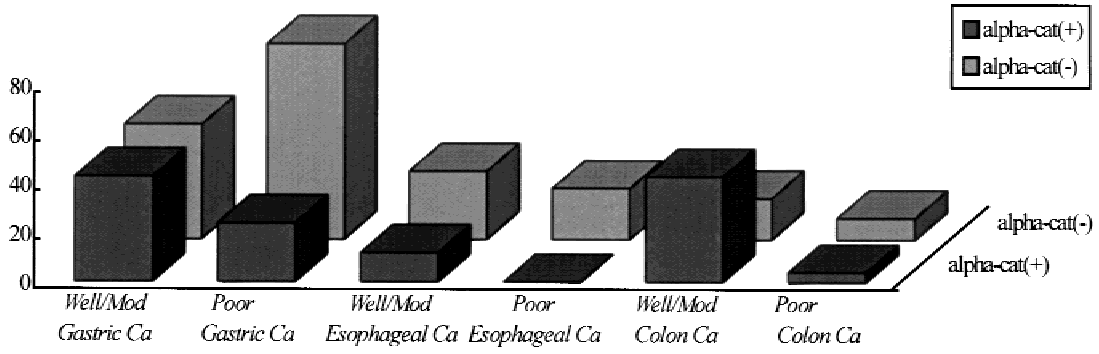

Fig. 3. Correlation of the level of α-catenin (α-cat) expression with the grade of differentiation in the gastric cancer (data from refs. 11, 16, 22), esophageal cancer (data from refs. 10, 16), and colorectal cancer (data from our study). Cancers have been divided according to the grade of differentiation as well/moderately and poorly differentiated and according to the level of α-catenin expression as preserved or α-cat(+) and reduced or α-cat(−).

ness of the cancer cell, while dysfunction of α-catenin, which connects the E-cadherin/catenin complex with the cytoskeleton, might alter the cell motility predisposing to metastasis.

No correlation was found in our study between decreased α-catenin expression in colorectal cancer and increased incidence of liver metastases. Generally, the frequency of haematogenous liver metastases is much lower than that of lymph node metastases and differentiated carcinomas, which usually preserve α-catenin expression, are more often associated with liver metastases than with lymph node metastases [24]. It is also possible that different mechanisms might be involved to the development of lymphogenous and haematogenous metastases.

Unlike previous studies, we also demonstrate that preserved α-catenin expression in colorectal cancer seems to be associated with longer survival rates and better prognosis. Down-regulation of E-cadherin has been reported to correlate with poor prognosis in colorectal cancer [6], but this finding has not yet been confirmed for the α-catenin. Two recent studies that investigated the α-catenin expression in colorectal adenocarcinomas and squamous cell carcinomas of head and neck respectively did not find any correlation of its down-regulation with patient survival [13,17]. Therefore, further studies with greater sample and longer follow up are needed in order to confirm our results and furter evaluate the prognostic value of α-catenin. Clearly, long-term follow-up evaluation of α-catenin-negative, well-differentiated, or Dukes A and B tumors would be of interest in order to further clarify the clinical value of α-catenin expression in colorectal carcinomas as a prognostic factor and a marker of differentiation and metastatic potential

It is noteworthy in our study, that colon cancer cells which expressed α-catenin tended to adhere to each other and build gland-like formations, while cancer cells that did not express α-catenin invaded sparsely and did not form close contacts. Our observation is in accordance with previous in vitro studies which have demonstrated that α-catenin expression is frequently down-regulated in highly invasive colon cancer cell lines and is associated with a nonepithelioid growth pattern [25,26]. Furthermore, correction of a defect in E-cadherin/α-catenin adhesion complex in a poorly differentiated colon cancer cell line with E-cadherin cDNA transfection increased cell–cell adhesion and retarded tumor cell migration on basement membrane [27]. Our findings are compatible with the idea that the detachment of colon cancer cells from the primary tumor, as a result of decreased E-cadherin and α-catenin expression, elicits the invasive phenotype, leads to metastasis and indicates unfavorable prognosis.

Controversy exists over the expression of the E-cadherin/catenin cell adhesion complex in the lymph node and liver metastases in comparison with the corresponding primary tumor. In our study, decreased α-catenin expression was found in 6 of 10 lymph node metastases and the pattern of expression was similar to that of the corresponding primary tumor. On the other hand, all 3 liver metastases showed preserved α-catenin expression and were originated from α-catenin(+) primary tumors. It has been reported that the intensity of α-catenin expression in liver metastases of colorectal adenocarcinomas [17] and nodal metastases of squamous cell carcinomas of the head and neck [13] is weaker in comparison with the primary tumors. In addition, 2 liver metastases of gastric carcinomas originated from an α-catenin(+) and α-catenin(−) primary tumor, respectively [11].

In accordance with our results, E-cadherin expression in lymph node metastases of colorectal carcinomas [28], gastric carcinomas [23,29] and squamous cell carcinomas of the head and neck [30] is frequently down-regulated, but it follows the pattern of expression of the primary tumor. Another study has reported the development of E-cadherin(+) lymph node metastases from E-cadherin(−) primary colorectal cancers [5]. Furthermore,

while 3 liver metastases preserve E-cadherin expression and derive from E-cadherin(+) gastric carcinomas [29], 6/14 liver metastases of colorectal cancers exhibit decreased E-cadherin expression in comparison with the primary tumors [4].

These observations appear difficult to be fully explained with the theory of cancer cell detachment. Our results indicate that the E-cadherin/α-catenin complex might be temporarily down-regulated, resulting in transient expression of the invasive phenotype. It has been postulated that temporary or local loss of E-cadherin or α-catenin expression might trigger the detachment of certain cancer cell subpopulation so as to initiate metastasis. At implantation sites, cancer cells might either reexpress the E-cadherin/α-catenin complex, or it may be that only E-cadherin(+)/α-catenin(+) cells have the ability to implant. Furthermore, our limited series of α-catenin(+) liver metastases could also suggest that the E-cadherin(+)/α-catenin(+) cancer cells are able to attach more easily to hepatocytes that express abundant E-cadherin, and therefore survive better in the new microenvironment [3,29].

CONCLUSIONS

Our study demonstrates an inverse association of the level of α-catenin expression with grade of differentiation, Dukes stage, and clinical outcome in colorectal carcinomas. Furthermore, the pattern of α-catenin expression in lymphogenous metastases appears to be different in comparison with liver metastases. However, larger samples are needed in order to confirm this finding. Overall, the α-catenin expression in metastases seems to correlate well with its expression at the corresponding primary tumor. In conclusion, the down-regulation of α-catenin expression in colorectal cancer seems to correlate with poor differentiation and high metastatic potential, but its prognostic value in colorectal cancer patients remains to be confirmed in the future.

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